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The role of biofertilizer on the growing efficiency of *Callisia fragrans* cultivated under open-air hydroponic conditions of the Ararat Valley

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ABSTRACT

Background: Invention of biofertilizers (BFs) is considered to be one of the most important achievements of modern agriculture. Although these microbial bioproducts have not found widespread usage in soilless production, there is an increasing trend of research studies on BFs in hydroponics.

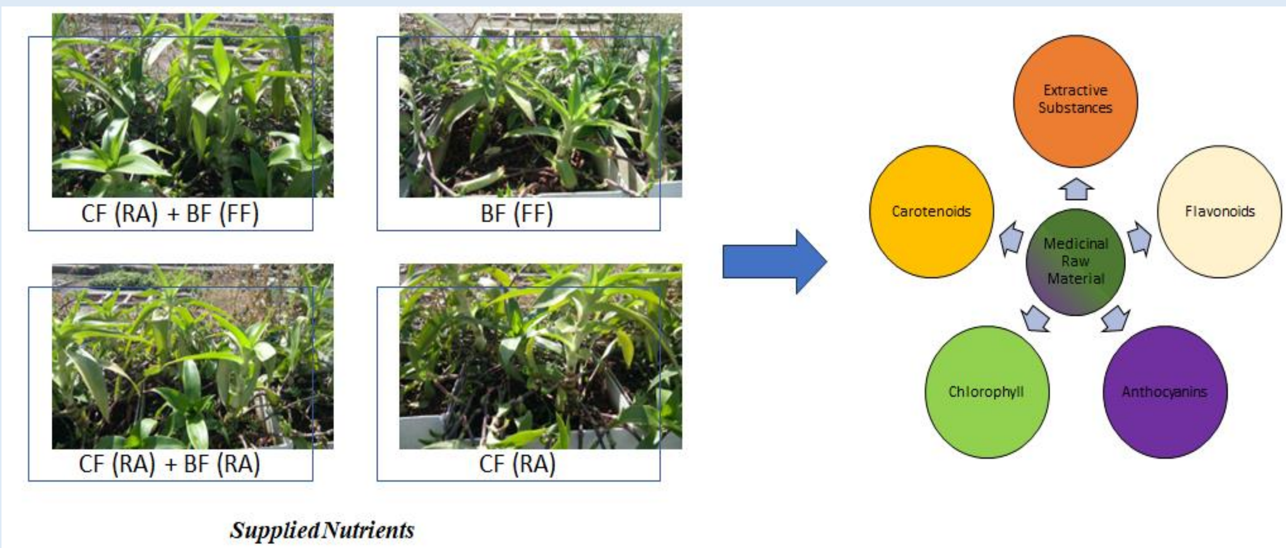
Objective: In consideration of the high potential of biofertilizers (BFs) in hydroponics, this study examines the impact of BF on the growth efficiency of the medicinal plant Callisia fragrans (Lindl.) Woodson (C. fragrans) under open-air hydroponic conditions in the Ararat Valley for the first time.

Methods: The plants were grown in a mixture of gravel and volcanic red slag. During the vegetation period, chemical fertilizer (CF), BF, as well as their different combinations were supplied to the plants. Plant's growth and development regularities were studied via biometrical measurements, which were done periodically during the growing process. Physiological (chlorophyll a, b, carotenoids), bio- and pharmacochemical (extractive substances, total amount of flavonoids, anthocyanins) analyses were done in the medicinal raw material. The obtained data were subjected to the statistical evaluation.

Results: At the end of the experimental period, the plants nourished with a combination of CF and BF, where the BF was supplied via foliar feeding (FF), as well as the plants given only CF through root application (RA), exhibited 2.9-3.5 times higher fresh overground biomass compared to the plants given BF (FF) alone. The application of BF (FF) with the combination in CF significantly increased the content of chlorophyll b and carotenoids in the leaves of plants compared to the BF (FF) variant. The maximum accumulation of anthocyanins and flavonoids of plants were mostly observed in the plants given the combination of CF and BF through the roots, as well as the ones given BF (FF) alone. Significant difference on the biosynthesis of extractive substances in the medicinal raw material between the variants has not been observed.

Conclusion: Summarizing the preliminary results of the experiments, it is becoming obvious, that the application of BFs has great potential in hydroponics in order to enhance growing efficiency of *C. fragrans*.

Keywords: *C. fragrans*, biofertilizer, chemical fertilizer, bioactive compounds, hydroponics, medicinal raw material



INTRODUCTION

The research of medicinal plants in terms of content of bioactive compounds (BAC) is highly important, because the presence and amount of them in the obtained medicinal raw material not only determine quality and potential therapeutic benefits of the plant, but also create basis for their isolation from medicinal raw material and further usage of them in medicine.

BAC are chemical substances, which have health benefits on the body, being contained in a plant or in a food even in small quantities [1]. Although the presence of any secondary metabolite in the plants comes genetically, the amount of the compounds may be easily changed under the influence of different environmental factors [2]. Biosynthesis of BAC may be affected by light [3-5], radiation [6], climate [7], drought stress [8-10], etc. Liu et al. (2016) showed, that the content of BAC of *Potentilla fruticose* L. is strongly affected by the region, where the plants were cultivated [11]. Another paper found that the phytochemical properties of *Allium fistulosum* L. may be improved through the optimal concentration of the applied nitrogen fertilizer [12]. González-Delgado et al. (2023) demonstrated beneficial impact of joint stresses of salinity and water on the accumulation of BAC in *Aloe vera* [13]. Biosynthesis of BAC in the plants may be promoted while cultivating them under soilless conditions [14].

Callisia fragrans (Lindl.) Woodson (C. fragrans) is a medicinal plant well-known in traditional medicine. Medicinal raw material of the plant is rich in BAC:

phenolic compounds [15-16], amino acids [15], organic acids [15, 17], anthocyanins [18], etc., and has therapeutic benefits, such as antioxidant [16-17], anti-bacterial [16], anti-inflammatory [17], anti-hypertensive [19], immunomodulating, stress-protective [15], anti-herpetic [20], radioprotective [21], etc. The research works on growth efficiency of *C. fragrans* under open-air hydroponic conditions of the Ararat Valley run successfully for years. The development of new technology for hydroponic cultivation, which comes to reduce the amount of chemical nutrients, at the same time enhance/promote the biosynthesis of BAC in plants is a core of the series of my current studies.

Biofertilizers (BFs) are eco-friendly substances, the application of which in sustainable agriculture is very perspective. There are various scientific studies, which approve positive effect of BFs on the accumulation of BAC in the plants [22-24]. Although the BFs are widely used in traditional farming, the popularity of their application in hydroponics began to increase only during the recent years. The reason of the rising interest in BFs in soilless culture may probably be related to the growing demand on organic products. As it is well known, in hydroponics chemical fertilizers (CFs) are mostly applicable, thus, the application of BFs in soilless production may be a very good alternative to chemical ones. One of the aspects that is important to take account while using BFs for soilless production is the strength of nutrient solution made of them: generally, the concentration of BFs is low, therefore the application of BFs in hydroponics requires long-term, gentle, and comprehensive studies.

There are well-known scientific studies that reference the application of these environmentally friendly bio-substances in hydroponics [25]. Although, the BFs are successfully used in agriculture, many authors reported the efficiency of their application in combination with CFs on various crops [26-28].

Taking into account the promising potential of using of BFs in soilless culture, in the frame of this study the role of application of a BF on the growing efficiency of *C. fragrans* cultivated under open-air hydroponic conditions of the Ararat Valley has been studied for the first time.

METHODS AND MATERIALS

The experiments were carried out in the experimental field of G.S. Davtyan Institute of Hydroponics Problems (National Academy of Sciences, Republic of Armenia).

Experimental Design: The saplings of *C. fragrans* were planted in special hydroponic pots (Ebb & Flow system) with 0.16 sqm nourishing surface. The planting density was 12 plants sqm^{-1}. As a substrate for growing, a mixture of gravel and volcanic red slag (1:1 volumetric ratio) was used. As a source of CF, Davtyan's nutrient solution [29] with a concentration of 0.5 N was applied. In the experiments, the working solution of BF "Ecobiofeed+" [30] developed by the scientists of "Armbiotechnology" SPS (National Academy of Sciences, Republic of Armenia) was used. The BF was supplied as through the root application (RA) as well as via foliar feeding (FF).

Based on the source of the supplied nutrients, as well as the way of nutrient uptake, the samples were divided into the following groups in the randomized experiment:

a) CF (RA) + BF (FF),
b) CF (RA) + BF (RA),
c) BF (FF),
d) CF (RA).

Medicinal Raw Material Processing: After harvesting the medicinal raw material, the leaves, stem and lateral sprouts of *C. fragrans* were separated and weighted. These parts were chopped into small pieces and air-dried under room conditions separately.

Biometrical Measurements: During the vegetation period, biometrical measurements were done periodically (once every 15 days). Plant's height, diameter of the stem, as well as the number of lateral sprouts were noticed.

Physiological, Bio- and Pharmacochemical Analyses: Contents of chlorophyll (a, b) and carotenoids in the fresh leaves of the plants were determined according to Lichtenthaler [31-32]. Total amount of flavonoids and content of extractive substances in the dried leaves and lateral sprouts were determined based on the recognized methods [33-36]. The number of anthocyanins in the fresh lateral sprouts was determined according to the known methods [37-38]. UV-VIS Cary 60 Spectrophotometer (Agilent) was used for the analyses. The above-mentioned analyses were conducted with several modifications. Procedures of the conducted analyses are presented in the following paragraphs.

Determination of Photosynthetic Pigments: 0.5 grams (accurately weighted) of sample were homogenized with 10 ml of 95% ethanol. The homogenized mass was centrifuged at 10,000 rpm for 15 minutes. 0.5 ml of supernatant was transferred into a flask. 4.5 ml of 95% ethanol was added to the flask. The obtained solution was used for determination of photosynthetic pigments under different wavelengths: chlorophyll a – 664 nm, chlorophyll b – 649 nm, carotenoids – 470 nm.

Determination of Total Amount of Flavonoids: 1 gram of analytic sample (particle diameter: 3-5 mm) was transferred into a 100 ml conical flask and 50 ml of 50% ethanol was added to it. The flask was connected to a reflux refrigerator and heated (50-60 ^{O}C) for 30 min on an electric plate. After cooling, the content of the flask was filtered into a 50 ml volumetric flask. The volume of the filtrate was made up to 50 ml with 50% ethanol (solution A). 2 ml of solution A was transferred into a 25 ml volumetric flask and made up to 25 ml with 96% ethanol (solution B). The optical density of solution B was measured at a wavelength of 370 nm. 96% ethanol was used as a standard. The content of total amount of flavonoids was calculated on quercetin.

Determination of Extractive Substances: For determination of extractive substances, solution A, which was made as described in the previous paragraph, was transferred into a previously weighted special bowl and evaporated on a water bath. After evaporation, the bowl with dried extracts was weighted.

Determination of Anthocyanins: 10 grams of sample (particle diameter: about 1.0 cm) were crushed and transferred into a 250 ml volumetric flask. 40 ml of 60% ethanol containing 1% hydrochloric acid was added to the crushed sample. The flask was covered and stored in the refrigerator for 24 hours at +4 ^{0}C. Afterward, the content of the flask was filtered and the volume of the filtrate was made up to 50 ml with 60% ethanol containing 1% hydrochloric acid (solution C). 1 ml of solution C was transferred into a 25 ml volumetric flask and made up to 25 ml with 60% ethanol containing 1% hydrochloric acid (solution D). Solution D was stored in the dark for 2 hours, afterward was measured for the optical density at a wavelength of 535 nm. 60% ethanol containing 1% hydrochloric acid was used as a standard.

Statistical Analyses: The obtained data underwent statistical analysis using the GraphPad Prism 6 statistical program. Tukey's multiple comparison test was used. The graphs were created with GraphPad Prism 6 and Microsoft Excel.

RESULTS AND DISCUSSION

Biometrical Measurements and Yield: According to the received data, the most intensive growth of the height of plants in different variants was observed during various time-periods. The maximum growth of the plants which were nourished with CF (RA) + BF (FF) was observed during the first part of the August. The plants, which were given CF alone, were grown effectively during the second part of the July. Meanwhile, CF (RA) + BF (RA) and BF (FF) variants provided the increasement of the growth of the plants' height during the first part of the September (Fig. 1A). Stem diameter was changed with another regularities. Maximum thickening of the stem of plants given CF (RA) + BF (FF) was observed during the second part of June. Meanwhile, the remaining variants showed the highest results of growth of stem diameter during the first part of July (Fig. 1B). Regarding the lateral sprouts, maximum growth of their number was observed during the August (Fig. 1C).

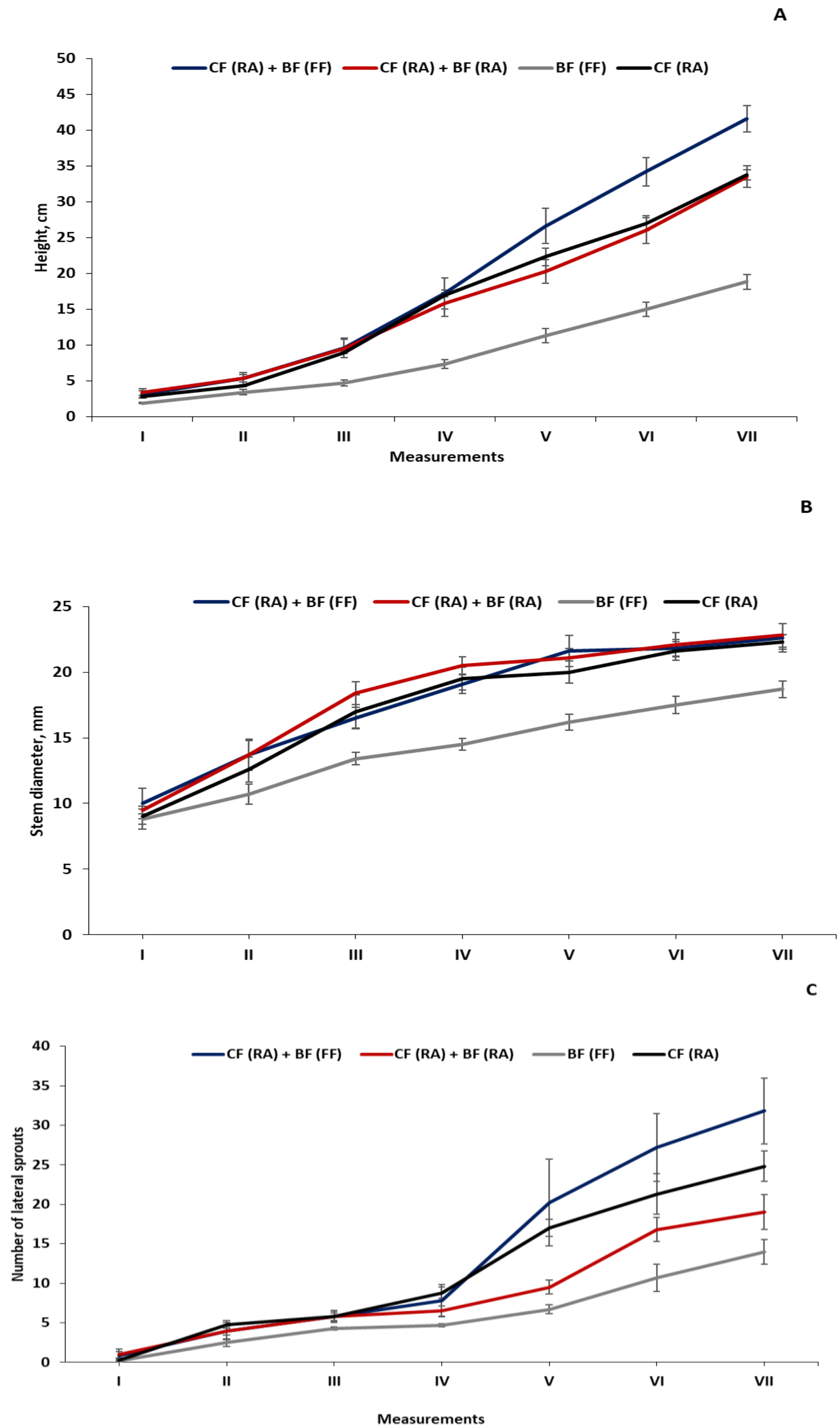


Figure 1. Growth parameters of *C. fragrans* cultivated with different nutrients supply under open-air hydroponic conditions of the Ararat Valley (Mean ± SEM): height (A), stem diameter (B), number of lateral sprouts (C)

At the end of the experimental period no significant difference was observed between the fresh overground biomass of plants given CF (RA) alone as well as combination of CF (RA) and BF (FF). By the fresh overground biomass, the both variants significantly (2.9-3.5 times) increased the ones given BF (FF) alone.

Physiological Analyses: According to the conducted statistical analyses, no significant differences were observed between chlorophyll a content in the tested variants (Fig. 2A). At the same time, the contents of chlorophyll b and carotenoids in the leaves of plants given BF alone through the FF were significantly decreased compared to the plants supplied CF (RA) + BF (FF) during the vegetation. The latter by the content of chlorophyll b and carotenoids exceeded the BF (FF) variant 1.9 and 1.6 times, accordingly (Fig. 1B, C).

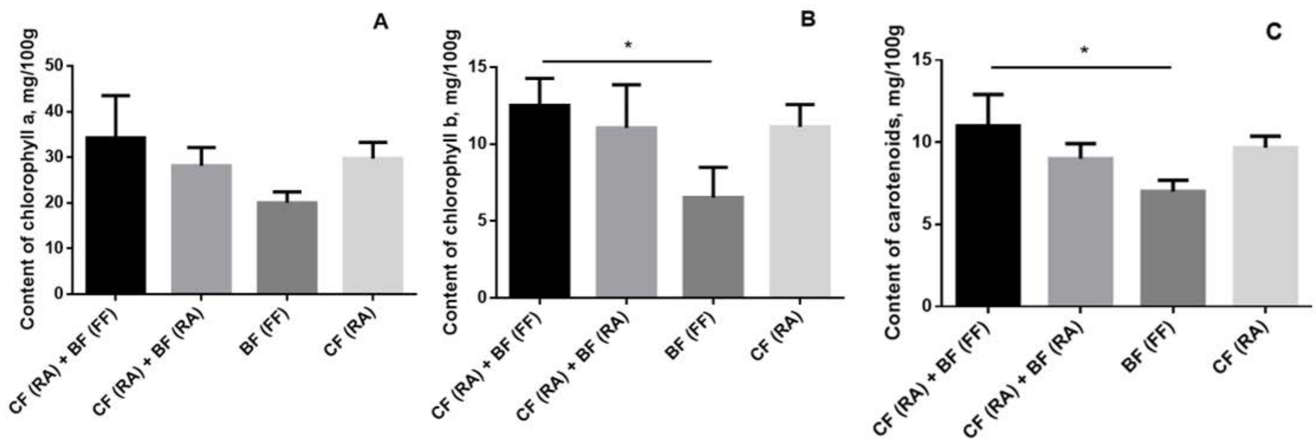


Figure 2. Content of chlorophyll a (A), b (B), and carotenoids (C) in the leaves of *C. fragrans* cultivated with different nutrients supply under open-air hydroponic conditions of the Ararat Valley (Mean ± SD)

Bio- and Pharmacochemical Analyses: The applied nutrient solutions affect the accumulation of total amount of flavonoids in the medicinal raw material of *C. fragrans*. The noticed differences were more obvious while analyzing the lateral sprouts of the plants. In the lateral sprouts, the application of BF alone through the FF, as well as via RA in combination with CF promoted the accumulation of total amount of flavonoids. With the content of flavonoids in the lateral sprouts, CF (RA) + BF (RA) exceeded the both CF (RA) + BF (FF) and CF (RA) variants 1.8 times. At the same time, the amount of flavonoids extracted from the lateral sprouts of plants which were nourished with BF alone via foliar application exceeded the above-mentioned variants by 1.9 and 2.0 times, accordingly. No significant differences were observed between CF (RA) + BF (RA) and BF (FF), as well as between CF (RA) + BF (FF) and CF (RA) variants (Fig. 3A). In the leaves of plants, significant difference on accumulation of flavonoids only between CF (RA) and BF (FF) was observed. Content of total amount of flavonoids obtained from the plants given only BF via FF exceeded the ones, which was supplied with only CF 1.7 times (Fig. 3B).

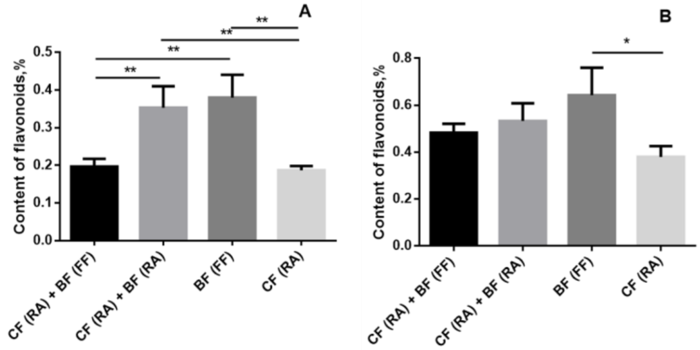


Figure 3. Content of total amount of flavonoids in the lateral sprouts (A) and leaves (B) of *C. fragrans* cultivated with different nutrients supply under open-air hydroponic conditions of the Ararat Valley (Mean ± SD)**.** Regarding the extractive substances, both in the leaves, as well as lateral sprouts of the plants no significant differences between the tested variants were observed (Fig. 4A, B).

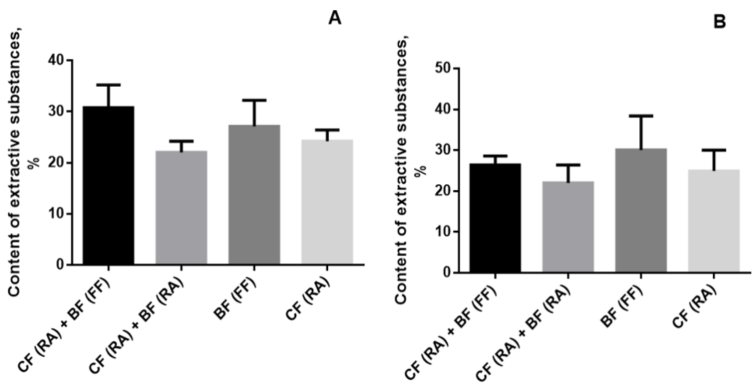


Figure 4. Content of extractive substances in the lateral sprouts (A) and leaves (B) of *C. fragrans* cultivated with different nutrients supply under open-air hydroponic conditions of the Ararat Valley (Mean ± SD)

As in the case of flavonoids, application of BF alone through FF, as well as via RA in combination with CF, created optimal conditions for the accumulation of anthocyanins in the lateral sprouts of *C. fragrans.* By the content of anthocyanins, the above-mentioned variants significantly (1.5 times) exceeded CF (RA) variant. No significant difference was observed between CF (RA) + BF (FF) and CF (RA) variants regarding the accumulation of anthocyanins in the medicinal raw material (Fig. 5).

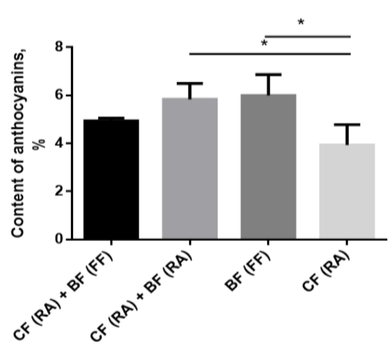


Figure 5. Content of anthocyanins in the lateral sprouts of *C. fragrans* cultivated with different nutrients supply under open-air hydroponic conditions of the Ararat Valley (Mean ± SD)

The similar results were obtained by other authors as well. Kolega et al. (2020) showed that soilless cultivation of sweet basil with nutrient solution and inoculation with *Azospirillum brasilense* affect positively the content of metabolites [27]. Vela Coyotl et al. (2018) demonstrated that the combined application of CF with BF through the FF increased the yield of faba bean grown in hydroponics [28]. Razmjooei et al. (2022) also demonstrated that the application of CF and BF together may enhance lettuce yield, photosynthetic and enzymatic activity, as well as the content of metabolites in hydroponics [39]. Summarizing the preliminary results of the conducted experiments, it may be concluded that the usage of BFs in hydroponics has great potential. Further development of the application methods of BFs in soilless culture to enhance targeted biosynthesis of BAC in medicinal raw materials would be an intriguing discovery in science.

CONCLUSION

The successful adaptation and application of BFs in hydroponics could serve as a viable alternative to CFs. Based on the results obtained from preliminary studies, it is becoming increasingly evident that the application of BFs in hydroponics holds great promise. Moreover, the method of applying BFs plays a pivotal role in achieving the desired medicinal raw material. For accumulation of *C. fragrans* biomass, foliar application of BF in combination with CF showed the best results, meanwhile for the accumulation of flavonoids and anthocyanins in the medicinal raw material application of BF through the roots in combination with CF, as well as application of BF alone via FF are more preferable. Application of BF alone did not promote the biosynthesis of chlorophyll b and carotenoids. The tested nutrient solutions did not affect significantly the biosynthesis of extractive substances.

List of Abbreviations: BAC, bioactive compounds; BF, biofertilizer; CF, chemical fertilizer; FF, foliar feeding; RA, root application.

Competing interests: The author declares that there are no competing interests.

Authors' contributions: AK designed the study, conducted the experiments, elaborated the obtained data, carried out the statistical analysis, wrote the manuscript.

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